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LETTER TO THE EDITOR

True Measles Cases Undetected by Reverse Transcription-PCR (RT-PCR): Effect of Genetic Variability on Assay Sensitivity Needs To Be Regularly Surveyed

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Considerable effort to eliminate measles has been made worldwide since the World Health Organization (WHO) elimination program was adopted in 2000 (1, 2). The laboratory confirmation of suspected cases is recommended by WHO since diagnosis based on clinical symptoms alone is unreliable. Detection of specific immunoglobulin M (IgM) is the standard method for the rapid laboratory confirmation of measles (3). The detection of measles virus by reverse transcription-PCR (RT-PCR) is gaining in importance in case confirmation. Increasing numbers of laboratories are introducing molecular detection using commercial kits.

Measles molecular epidemiology is a tool recommended by WHO for tracking importations and linking cases and for demonstrating the absence of sustained measles transmission in different countries.

Here we describe how measles molecular surveillance allowed the identification of virus single nucleotide polymorphisms (SNP), which led to false negatives using a commercial RT-PCR kit. In November 2018, two samples from clinically suspected measles patients were sent to the laboratory of Mamoudzou hospital (Mayotte). These samples tested negative for measles virus RNA using an FTD fever and rash kit from Fast Track Diagnostics. The primers and probe of this kit target the gene coding for the hemagglutinin (H) protein. The samples were then forwarded to the Measles National Reference Center (NRC), Caen, France, for further investigation. Here, measles viruses were detected in both samples using the in-house test provided by the U.S. Centers for Disease Control (CDC) (4). The cycle threshold (C_T) values produced in real-time RT-PCR for the two samples were 23.2 and 25.5, respectively. The C_T value is associated with the amount of PCR product in the reaction mixture. The lower the C_T value, the more PCR product is present. For verification, the samples were retested using an FTD fever and rash kit, and the negative results were confirmed while all negative and positive controls worked well and proved the good function of the reactional mix. The viruses were identified as belonging to genotype B3, and they clustered separately from other B3 viruses circulating in France during the same period (Fig. 1). The complete H gene of Mayotte strains was sequenced using the 4 original primer pairs reported in Table 1. Analysis of the sequences obtained showed SNP compared to other B3 measles

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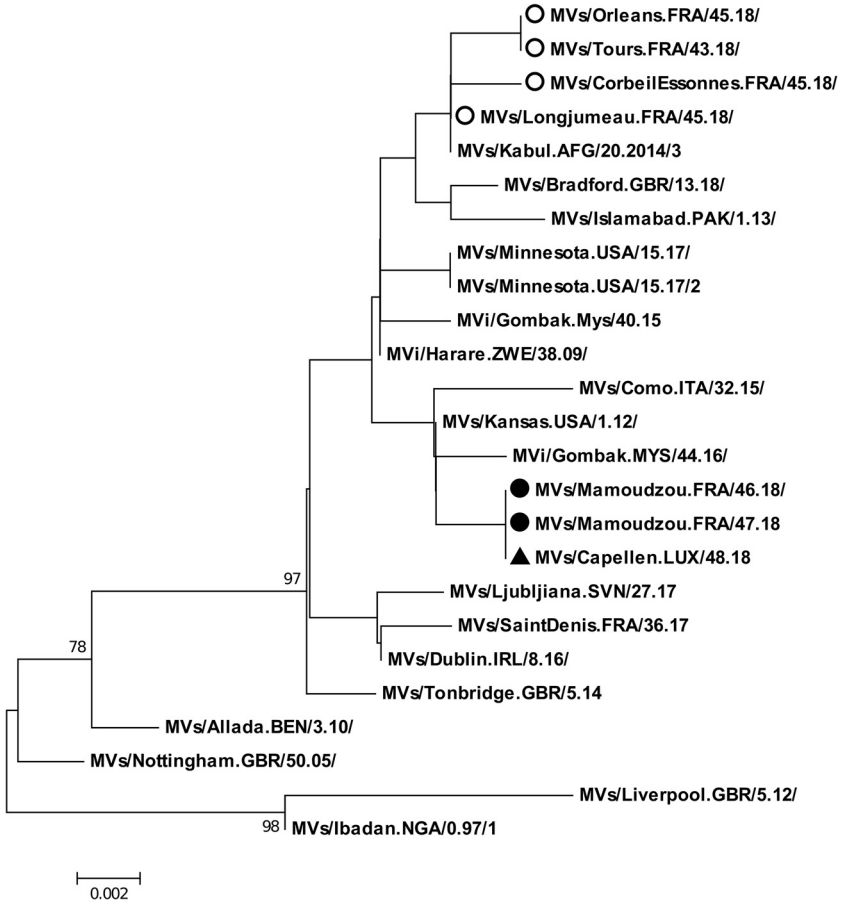

FIG 1 Phylogenetic analysis of the N-450 sequences of B3 measles strains. The figure shows the phylogenetic relationship between measles viruses isolated in Mayotte (2 strains; identified with filled circles), in Luxembourg (1 strain; identified with filled triangle), and in different regions in France (4 strains; marked with empty circles) and 17 reference strains identified as B3-named strains in MeaNS. The tree was constructed with Mega6 software using the NJ K2P method. Bootstrap values are shown when >70.

viruses found in GenBank (Fig. 2). The sequencing of the 450 nucleotides that code for the C-terminal N gene (N 450) defining the measles virus genotype and H gene sequences from both samples were submitted to Measles Nucleotide Surveillance Database MeaNS (MeaNS identifiers [IDs] 134661, 134662, 138586, and 138587) and to GenBank (accession numbers MK733315, MK733316, MN052911, and MN052912). Only one other sequence identical to our N 450 sequences was identified in MeaNS (5). This sequence had been detected in a measles patient returning from Madagascar to Luxembourg (MeaNS ID 133353).

A measles outbreak involving 19,539 cases that started in October 2018 and

TABLE 1 Sequences of primers used to amplify and sequence the complete H gene of measles virus

Name of primer	Sequence
MvF6670Fw	AAT CAA GAC CCT GAC AAG ATC CT
MvH7489Rv	GCT TTT ATG GAT CTC CGC GGT GT
MvH7254Fw	GTG CAA GAT CAT CCA CAA TGT CAC C
MvH8262Rv	GTT GGG GAT TTC CAG ACA
MvH7905Fw	GAG GTT ACA ATG TGT CAT CTA
MvH8810Rv	GGT TGG AAC TGA GTT TGA CAT
MvH8673Fw	ACA CAT TGG AGT GGA TAC CGA
MvL9391Rv	AGG CGG TGC TTG ATG TTC TG

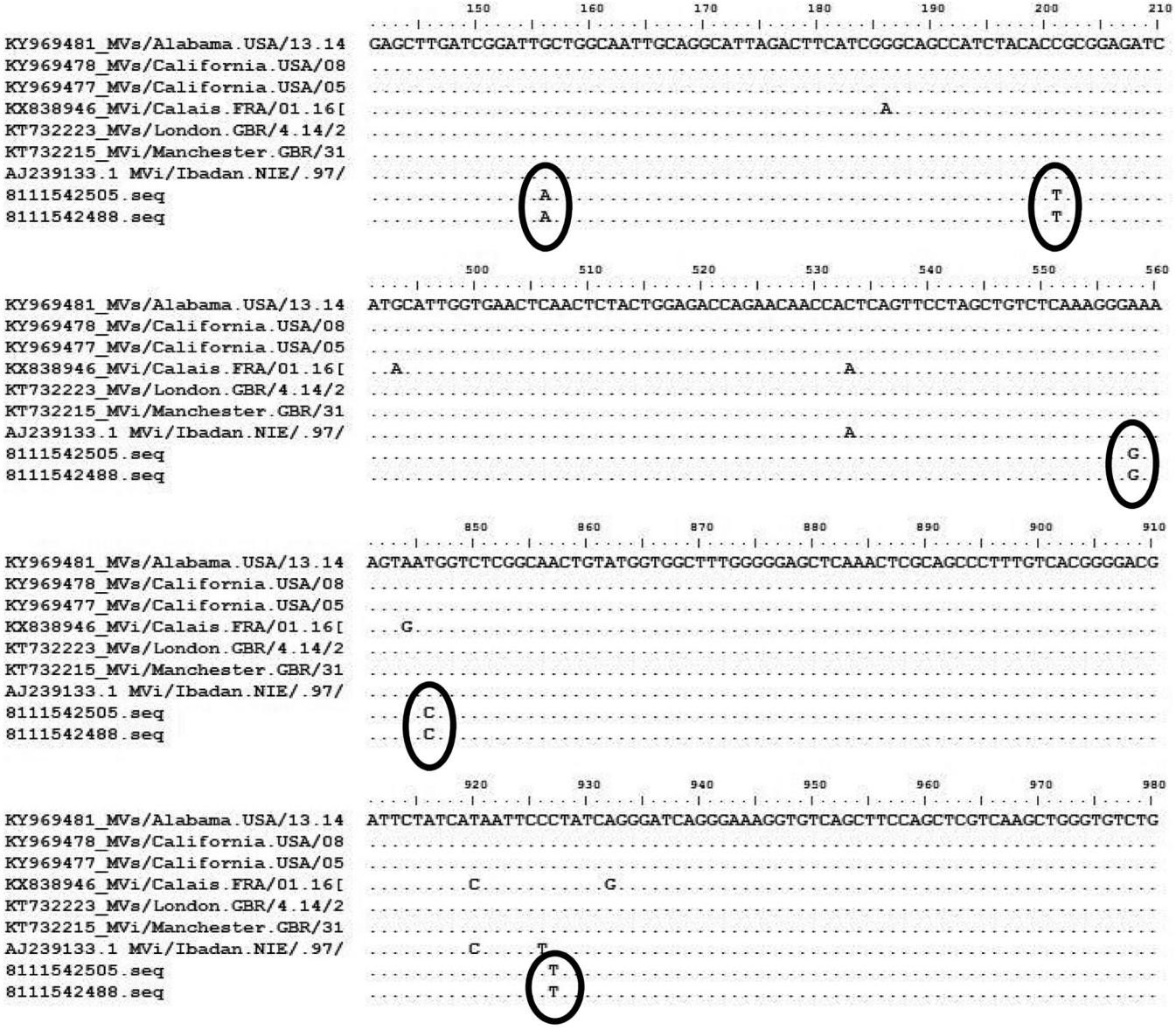

FIG 2 Alignment of complete H gene sequences (1,854 nucleotides) of genotype B3 measles viruses with a focus on regions where mutations were identified. The alignment was constructed with BioEdit software using the two B3 strains from Mayotte (Mamoudzou) and 7 other B3 strains from GenBank identified from 1997 to 2016. The sequences are identified with the MeaNS name and GenBank accession number when available. Mutations in the Mamoudzou strains are situated at positions 156, 201, 558, and 927.

lasted until January 2019 was reported from Madagascar (6). Mayotte Island is situated in the Indian Ocean next to Madagascar, which may explain the match between Mamoudzou sequences and the measles sequence found in a patient returning from Madagascar.

Measles virus is known to have low genetic variability, with only a few SNP reported within each lineage (7). However, since even a single mutation may seriously impact molecular diagnosis, as shown in the present report, it is essential to continuously monitor measles genetic variability to ensure that no cases are missed and to use validated molecular assays and protocols. Some authors experiencing quantitative PCR (qPCR) escape variants suggested previously that using two distinct PCR targets simultaneously allows investigators to avoid false negatives (8). This report underscores the importance of careful interpretation of negative RT-PCR results, which should never be the sole basis for ruling out measles in a suspected case (3).

Data availability. Sequences were deposited in GenBank under accession numbers MK733315, MK733316, MN052911, and MN052912.

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